

Cof Sub D11
37. (Amended) A recombinant herbicide tolerance nucleic acid made by the method of claim 1 or 4.

C5 Sub D12
61 38. (New) The method of claim 1 or 4, further comprising isolating or recovering at least one identified recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance.

No New Matter

The amendments to the claims present no new matter. The above amendments are presented to clarify and more particularly address aspects of the invention. In addition, the amendments are provided to correct minor defects in antecedence and to provide a more consistent lexicography. Support for the claims as amended is found throughout the specification and claims as originally filed.

REMARKS

With this response, claims 1-38 are pending. Claims 1-37 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Applicants traverse, to the extent that the rejections are applied to the amended claims. In addition, Claims 1-37 were rejected under 35 U.S.C. §103(a) over Khosla in view of Subramanian, in some cases, in combination with various additional references. Applicants traverse each of these rejections for the reasons noted below.

CLAIMS 1-37 ARE NOT INDEFINITE

Claims 1-37 were rejected as allegedly indefinite. Each of the alleged grounds is addressed in turn as follows. Claims 1-37 were rejected as allegedly being indefinite for failing to recite a final process step that clearly relates back to the claim preamble. Applicants have amended the claims for clarity, overcoming the rejections. The claims have been amended to indicate that the methods relate to the identification of recombinant herbicide tolerance nucleic acids that can confer tolerance to an herbicide upon a cell. In step (iii) of the method embodied, e.g., in claim 1, a library of recombinant nucleic acids is screened "to identify at least one recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance to a cell," clearly relating back to the preamble.

Claims 1-37 were also alleged to be indefinite with respect to the term “obtaining.” As indicated above, the claims have been amended to clarify that the method, e.g., as embodied in claim 1, is directed to “identifying” a recombinant herbicide tolerance nucleic acid.

Claims 1-37 were also rejected as allegedly indefinite over the recitation of the term “derived.” Applicants traverse the rejection. As amended, e.g., claim 1, recites “a plurality of nucleic acid segments, which nucleic acid segments are derived from a plurality of variant forms of one or more parental nucleic acids.” Taken in light of the statement that the term a ‘Nucleic acid derived from a gene’ refers to a nucleic acid for whose synthesis the gene, or a subsequence thereof, has ultimately served as a template,” (page 16, lines 15-16) it can be unequivocally concluded that the plurality of nucleic acid segments have been synthesized, e.g., by transcription, replication, amplification, etc., using the variant forms (or subsequences thereof) of one or more parental nucleic acids as a template.

In addition, claims 1-37 were rejected as indefinite over the recitation of “nucleic acid encodes …activity.” While the Applicants disagree, in light of the clear definition provided on p. 10, lines 1-4: “An ‘activity’ of a protein (or, an ‘activity’ encoded by a nucleic acid) can include a catalytic (i.e., enzymatic) activity, an inherent physical property of the encoded protein (such as susceptibility to protease cleavage, susceptibility to denaturants, ability to polymerize or depolymerize), or both,” the claims have been amended, merely to clarify, without altering the scope of, the claim, that the nucleic acid encodes “a polypeptide with an activity.”

Claims 1-37 were further rejected as allegedly indefinite due to the recitation of the phrase “wherein the plurality of variant forms differ from each other in at least one nucleotide.” This clause has been removed from the claims as amended, as it is redundant and provides no further limitation beyond the term “variant forms” for which there is ample and clear definition throughout the specification and claims as filed, e.g., p. 10, lines 17-28; pp. 19-20.

Claims 1-37 were allegedly indefinite over the recitation of the term “identify.” Applicants traverse. The term “identify” is used in the art, and throughout the present application as synonymous with the term “detect.” In the context of the claim language, screening is performed to “identify,” i.e., “detect” nucleic acids with certain specified properties. Thus identification is a product of a screening process, and can not be considered to encompass merely

mental steps divorced from properties, e.g., sequence, structure, activity of an encoded polypeptide or protein. To further clarify the distinction between "identify" and e.g., "isolate" or "recover," new claim 38, has been provided to actively recite this optional process step.

Claim 30 was rejected as indefinite as allegedly unclear as to how the claim further limits claim 1. Claim 30 has been amended in accordance with the helpful suggestions of the Examiner to clearly indicate how the process steps of claim 30 relate to those of claim 1.

For the reasons noted above, Applicants respectfully request that the rejections of claims 1-37 under 35 U.S.C. §112, second paragraph, be withdrawn.

CLAIMS 1-7, 9-10, 12-20, 23-33, AND 35-37 ARE NOT OBVIOUS OVER KHOSLA IN VIEW OF SUBRAMANIAN.

Claims 1-7, 9-10, 12-20, and 35-37 were rejected as obvious over Khosla et al. [United States Patent Number 5,521,077] in view of Subramanian et al. (1997) J. Industrial Microbiology and Biotechnology 19:344-349. Applicants traverse for the reasons noted below.

Applicants respectfully submit that the Office Action fails to make a proper *prima facie* case of obviousness. Furthermore, on the basis of the cited references, such a case cannot be made.

According to the M.P.E.P. §2143:

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F. 2d 488, 20 USPQ2d 1438 (Fed Cir. 1991).

With respect to the claimed invention, none of these criteria is met. Claims 1-37 are drawn to a method of identifying a recombinant herbicide tolerance nucleic acid which can confer tolerance to an herbicide upon a cell in which the recombinant herbicide nucleic acid is present. The claimed method involves three steps: 1) providing a plurality of nucleic acid segments, which nucleic acid segments are derived from a plurality of variant forms of one or more parental nucleic acids, wherein, in alternative embodiments, the parental nucleic acids

encode a polypeptide possessing or lacking an herbicide tolerance activity; 2) recombining the segments to produce a library of recombinant nucleic acids; and 3) screening the library to identify at least one recombinant nucleic acid encoding a polypeptide with an activity that confers herbicide tolerance to a cell.

The Office Action alleges that the Khosla reference provides all of the limitations of the claimed invention except that “Khosla et al. do not teach or suggest employing their method to obtain or identify nucleic acid encoding an activity which confers herbicide tolerance to a cell. It is alleged that Subramanian et al. disclose the remaining limitations of the claims, and furthermore, provide the motivation to combine the references. Applicants disagree.

Khosla et al. teach a method of producing protein variants in which two discrete pools of mutants (typically, induced variants) are cloned into distinct vectors to produce independent “donor” and “recipient” substrate pools. The two libraries are transduced into separate populations of cells, i.e., “donor” and “recipient” cell populations. The “donor” library is amplified, recovered, and then transduced into the “recipient” cell population, where the “donor” and “recipient” vectors recombine to produce a library of “recombinant” protein variants, which are selected or screened for a desired characteristic. No discussion is provided regarding the suitability of herbicide tolerance as a desired characteristic, nor are any potential target nucleic acids suggested as potential substrates.

In contrast, Subramanian et al. relate to methods of screening natural populations, e.g., of microorganisms to discover new nucleic acids that confer herbicide tolerance on a cell, or plant in which the nucleic acid is present. Specifically, Subramanian provide methods of screening microorganisms, in particular bacterial strains, for desired activities. Three potential sources of microorganisms are provided: 1) soil enrichment; 2) known strains, e.g., as available from ATCC; and 3) anaerobic strains of bacteria. In addition, the possibility of screening for “different oxidative enzymes” from any of these sources is disclosed. The reference describes the utility and diversity of activities, and the nucleic acids that can confer such activities, present in naturally occurring microorganisms and eukaryotes, such as certain plant strains. The methods described illustrate the identification of natural strains of microorganisms with activities that confer herbicide tolerance, as exemplified by the ability of the microorganism to grow in

culture in the presence of the test herbicide. At no point are the nucleic acids encoding the polypeptides that confer the desired activity identified or isolated from the microorganisms.

Thus, while Subramanian et al. suggest that providing more options for metabolizing enzymes to confer herbicide selectivity in crops would be desirable, the methods which they disclose for so doing involve screening naturally occurring organisms, primarily microorganisms, for herbicide tolerance. Nothing in the disclosure suggests that additional methods might be of any use. No mention of producing variants, by any method, is suggested or taught. Indeed, the possibilities are strictly limited to the screening of organisms, and, indeed, to certain limited means of obtaining the organisms to be screened, e.g., soil enrichment, known strains, and/or anaerobic activities of the above.

The Action alleges that Table 1 of Subramanian discloses "that herbicide tolerance may result from altered target proteins," thereby suggesting the use of parental nucleic acids encoding such proteins in the method of Khosla et al. However, in the context of Table 1 and the supporting disclosure, "altered" merely refers to differences found between two naturally occurring species, and transferred, e.g., by transgenesis, from one species to another. In no way does the reference disclose or infer the introduction of mutations or "variations" as a means of changing the properties of a polypeptide encoded by a nucleic acid. Thus, nothing in the disclosure of Subramanian would lead a practitioner of ordinary skill in the art, absent the teaching of the subject application, to search for a method of mutating specific subsets of substrate nucleic acids to generate recombinant nucleic acids that encode proteins with herbicide tolerance activities. Indeed, as Subramanian et al. make no mention of isolating specific substrate nucleic acids, it is unclear which nucleic acids could be subject to a mutation or recombination processes to generate a recombinant library useful for screening. There is simply no logical nexus between the cited references.

Accordingly, no motivation can be found, in either the Khosla or the Subramanian reference, or in the knowledge available to one of ordinary skill in the art at the time the invention was made, absent the disclosure of the present invention, to combine the teachings of the disparate references.

Similarly, no expectation of success can be inferred from the cited references, separately or in combination as no disclosure is provided in either reference relating to the

selection of suitable substrate nucleic acids. Indeed, the disclosure of Subramanian would discourage any expectation of success, teaching away from the possibility of engineering crop selectivity in unspecified nucleic acids encoding unknown targets, i.e., unknown target sites, *see, e.g.*, p. 345, second column, lines 1-4.

Finally, even if a motivation to combine the cited references could be found, the references, individually and/or in combination, fail to teach all of the limitations of the claimed invention. For example, claim 1 (and claim 4) from which all the other claims depend, in step (i) recites “providing a plurality of nucleic acid segments.” The methods of Khosla et al. involve providing intact genes encoding protein variants as the substrates for recombination. Similarly, step (iii) of the claimed invention involves “screening to identify at least one recombinant herbicide tolerance nucleic acid...” Neither reference discusses identification of recombinant nucleic acids encoding polypeptides with an herbicide tolerance activity. On the one hand, Khosla is silent on the subject of herbicide tolerance, and on the other hand, Subramanian makes no mention of the identification of particular nucleic acids, and/or their isolation (*e.g.*, claim 38) of the present invention. In addition, neither reference provides any indication that variant forms of a parental nucleic acid that encodes a polypeptide lacking herbicide tolerance activity, *e.g.*, as recited in claim 4, are suitable, in any way, as substrates for the identification of recombinant nucleic acids that confer herbicide tolerance to a cell. Thus numerous elements of the claimed invention cannot be found or inferred in the cited references, whether considered individually or in combination.

As the above discussion demonstrates, none of the criteria required to establish a *prima facie* case of obviousness are met with respect to the claimed invention based on the cited references. Indeed, no such case can be established based on the teachings of the cited references. Applicants, therefore, respectfully request that the rejection be withdrawn.

CLAIM 8 IS NOT OBVIOUS OVER KHOSLA IN VIEW OF SUBRAMANIAN FURTHER IN VIEW OF KREBBER

Claim 8 was rejected as allegedly obvious over Khosla and Subramanian further in view of Krebber. Applicants traverse. The Office Action alleges that given the combination of Khosla and Subramanian, an ordinary artisan would have been motivated to produce induced

variants by the methods disclosed in Krebber. In fact, this cannot be the case, as no motivation exists to introduce mutations, i.e., variations, in a parental nucleic acid based on the disclosures of Khosla and Subramanian, whether considered individually or in combination, as described above. Applicants respectfully submit that the rejection should be withdrawn.

CLAIM 21-22 ARE NOT OBVIOUS OVER KHOSLA IN VIEW OF SUBRAMANIAN FURTHER IN VIEW OF PADGETTE

Claims 21 and 22 were rejected as allegedly obvious over Khosla and Subramanian further in view of Padgette. Applicants traverse. The Office action alleges that given the combination of Khosla and Subramanian, it would have been *prima facie* obvious to an ordinary artisan to screen variant nucleic acids in Mpu+ and/or AroA- bacteria. However, absent the disclosure of the subject application, this would not, in fact, be the case. No motivation to combine the teachings of Khosla and Subramanian exists. Furthermore, even in combination, Khosla and Subramanian fail to teach the limitations of the claimed invention. Nothing in Padgette can be shown to remedy these deficiencies. Accordingly, Applicants respectfully request that the rejection be withdrawn.

CLAIM 34 IS NOT OBVIOUS OVER KHOSLA IN VIEW OF SUBRAMANIAN FURTHER IN VIEW OF AONO

Claim 34 was rejected over Khosla and Subramanian further in view of Aono. Applicants traverse. Claim 34 was rejected on the basis of a combination of the teachings of Khosla and Subramanian as applied to, e.g., claims 1 and 4, from which 34 is dependent. As discussed above, Khosla and Subramanian, individually or in combination, fail to teach the limitations of these claims. Nothing in the Office Action indicates how the teachings of Aono can be applied to remedy these deficiencies, or further to meet the limitations of claim 34, considering that the Khosla and Subramanian references fail to teach the limitations of the independent claims from which claim 34 depends. Applicants respectfully request that the rejection be withdrawn.

CONCLUSION

In light of the amendments and remarks presented above, Applicants believe that claims 1-38 are now in condition for allowance. In the event that any issues of substance remain, **APPLICANTS HEREBY REQUEST AN EXAMINER INTERVIEW PRIOR TO PREPARATION OF ANY ADDITIONAL WRITTEN ACTION BY THE EXAMINER.**

Please feel free to call the undersigned to arrange for an Examiner's interview or to discuss the status of the application.



Respectfully submitted,


Gwynedd Warren, Ph.D.
Reg. No. 45,200

LAW OFFICES OF JONATHAN ALAN QUINE
P.O. BOX 458
Alameda, CA 94501
(510) 337-7871
Fax (510) 337-7877



Marked Copy of Amended Claims for
USSN 09/373,333

1. (Amended) A method of [obtaining] identifying a recombinant herbicide tolerance nucleic acid which can confer tolerance to an herbicide upon a [plant] cell in which the recombinant herbicide tolerance nucleic acid is present, the method comprising:

(i) [recombinating] providing a plurality of [variant forms of one or more parental] nucleic acid[,] segments, [wherein the plurality of variant forms comprises] which nucleic acid segments are derived from [the parental nucleic acid, wherein the] a plurality of variant forms of one or more parental nucleic acids, wherein one or more of the parental nucleic acids encode[s] a[n] [herbicide] polypeptide with [tolerance activity, or can be shuffled to encode] an herbicide tolerance activity[,]; [and wherein the plurality of variant forms differ from each other in at least one nucleotide, to produce a library of recombinant nucleic acids;]

(ii) recombinating the plurality of nucleic acid segments to produce a library of recombinant nucleic acids; and,

(iii) screening the library to identify at least one recombinant herbicide tolerance nucleic acid [wherein the recombinant herbicide tolerance nucleic acid]which encodes a polypeptide with an activity that confers herbicide tolerance to a cell.

2. (Amended) The method of claim 1, wherein the at least one recombinant herbicide tolerance nucleic acid encodes a polypeptide with a distinct or improved herbicide tolerance activity compared to [the] a parental nucleic acid.

3. (Amended) The method of claim 1, wherein [the one or more] a plurality of parental nucleic acids encode [s an] polypeptides with herbicide tolerance activit[y]ies[.], which herbicide tolerance activities are the same or different between polypeptides.

4. (Amended) [The] A method of [claim 1, wherein the parental] identifying a recombinant herbicide tolerance nucleic acid[s] [do not encode herbicide] which can confer tolerance [activity, wherein recombinating the plurality of variant forms

provides a] to an herbicide upon a cell in which the recombinant herbicide tolerance nucleic acid [which encodes an herbicide tolerance activity.] is present, the method comprising:

(i) providing a plurality of nucleic acid segments, which nucleic acid segments are derived from a plurality of variant forms of one or more parental nucleic acids, wherein the one or more parental nucleic acids encode a polypeptide lacking herbicide tolerance activity;

(ii) recombining the plurality of nucleic acid segments to produce a library of recombinant nucleic acids; and,

(iii) screening the library to identify at least one recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance to a cell.

5. (Amended) The method of claim 1 or 4, wherein [the] one or more parental nucleic acids encode[s] a polypeptides which [is] are functionally or structurally similar to an herbicide target protein.

6. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms [of the parental nucleic acid] comprises allelic or interspecific variants of one or more [the] parental nucleic acids.

7. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms [of the parental nucleic acid] is produced by synthesizing a plurality of nucleic acids homologous to the one or more parental nucleic acids.

8. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms [of the parental nucleic acid] is produced by error-prone transcription of the one or more parental nucleic acids or by replication of the parental nucleic acid in a mutator cell strain.

9. (Amended) The method of claim 1 or 4, wherein the one or more parental nucleic acids encode[s] a polypeptide or polypeptide fragment selected from the group

consisting of: a P450 monooxygenase polypeptide, a glutathione sulfur transferase polypeptide, a homoglutathione sulfur transferase polypeptide, a glyphosate oxidase polypeptide, a phosphinothricin acetyl transferase polypeptide, a dichlorophenoxyacetate monooxygenase polypeptide, an acetolactate synthase polypeptide, a protoporphyrinogen oxidase polypeptide, a 5-enolpyruvylshikimate-3-phosphate synthase polypeptide, and a UDP-N-acetylglucosamine enolpyruvyltransferase polypeptide.

12. (Amended) The method of claim 1 or 4, wherein the library comprises at least one recombinant nucleic acid produced by recombining a plurality of variant forms of [a] one or more parental nucleic acids selected from the group consisting of:

a P450 monooxygenase nucleic acid, a homoglutathione sulfur transferase nucleic acid, a glutathione sulfur transferase nucleic acid, a glyphosate oxidase nucleic acid, a phosphinothricin acetyl transferase nucleic acid, a dichlorophenoxyacetate monooxygenase nucleic acid, a acetolactate synthase nucleic acid, a enolpyruvylshikimate-3-phosphate synthase nucleic acid, and a UDP-N-acetylglucosamine enolpyruvyltransferase nucleic acid.

13. (Amended) The method of claim 1 or 4, wherein the screening comprises a step selected from the group consisting of:

- (a) screening for oxidation of [the]an herbicide;
- (b) screening for glutathione conjugation to [the]an herbicide or to a metabolite of [the]an herbicide; and,
- (c) screening for homoglutathione conjugation to [the]an herbicide or to a metabolite of [the]an herbicide.

14. (Amended) The method of claim 1 or 4, wherein the library of recombinant nucleic acids is present in a population of cells.

23. (Amended) The method of claim 1 or 4, the method further comprising screening the library for one or more additional activity that confers tolerance to one or more additional herbicides.

24. (Amended) The method of claim 1 or 4, wherein the step of recombining is performed in a [plurality] population of cells.

25. (Amended) The method of claim 24, further comprising:

(a) recombining [DNA from the plurality of cells that encode] at least one nucleic acid encoding acid encoding a polypeptide with an herbicide tolerance activity [with a second library of DNA fragments, at least one of which undergoes recombination with a segment in a nucleic acid present in the cells to produce recombinant cells, or recombining DNA between the plurality of cells that encode herbicide tolerance activity to produce modified cells.] by introducing a second plurality of nucleic acid segments into the population of cells, such that at least one introduced nucleic acid segment recombines with a nucleic acid present in a member of the population of cells to produce modified cells or recombining at least one nucleic acid that encodes a polypeptide with an herbicide tolerance activity between at least two members of the population of cells to produce modified cells.

26. (Amended) The method of claim 25, further comprising:

(b) recombining and screening the recombinant or modified cells to produce further [recombined] modified cells that have evolved [additionally] an additional distinct or improved herbicide tolerance activity.

27. (Amended) The method of claim 26, further comprising:

repeating (a) or (b) until the further [recombined] modified cells have acquired [additionally] an additional distinct or improved herbicide tolerance activity.

28. (Amended) The method of claim 1 or 4, wherein the method further comprises:

[(iii)] (iv) recombining at least one recombinant herbicide tolerance nucleic acid with an [further] additional nucleic acid, wherein the [further] additional nucleic acid is the same or different from one or more of the [plurality of the] variant forms of (i), to produce an additional further library of recombinant nucleic acids; and

[(iv)] (v) screening the [further] additional library to identify at least one [further] additional recombinant herbicide tolerance nucleic acid that encodes a [further] polypeptide with an additional improved herbicide tolerance activity compared to a non-recombinant herbicide tolerance [gene;] nucleic acid; and, optionally, repeating [(iii)] (iv) and [(iv)] (v).

29. (Amended) The method of claim 28, wherein the [further] additional recombinant herbicide tolerance nucleic acid encodes two or more distinct or improved herbicide tolerance activities.

30. (Amended) The method of claim 1 or 4, wherein the library of recombinant nucleic acid is present in bacterial cells and the [method] screening of step (iii) comprises:

pooling [multiple] a plurality of cells each comprising a separate member of the library [members] produced in step (ii);

screening the resulting pooled [library members] cells for a recombinant herbicide tolerance nucleic acid that encodes a polypeptide with a distinct or improved herbicide tolerance activity compared to a non-recombinant herbicide tolerance nucleic acid; and,

[cloning the distinct or improved recombinant herbicide tolerance]
isloating nucleic acid encoding the polypeptide with the distinct or improved
herbicide tolerance activity.

31. (Amended) The method of claim [2] 1 or 4, [wherein the] comprising screening for a distinct or improved herbicide tolerance activity, which activity is selected from the group consisting of: an increase in ability to metabolize [the] an herbicide; an increase in the range of herbicides to which the activity confers tolerance; an increase in expression level compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to inhibition by [the] an herbicide compared to that of an activity encoded by the parental nucleic acid; a decrease in susceptibility to protease cleavage compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to high or low pH levels compared to that of a polypeptide

encoded by the parental nucleic acid; a decrease in susceptibility to high or low temperatures compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in toxicity to a host plant compared to that of a polypeptide encoded by the selected nucleic acid; and any combination of two or more activities selected [thereof] therefrom.

32. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant.

33. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and testing the resulting transduced plant for tolerance to the herbicide.

34. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and breeding the plant with [a separate] another plant strain of the same species, [followed by selection of resulting] and selecting resulting offspring for tolerance to [the] an herbicide.

35. (Amended) A library of recombinant nucleic acids made by the method of claim 1 or 4.

37. (Amended) A recombinant herbicide tolerance nucleic acid made by the method of claim 1 or 4.

38. (New) The method of claim 1 or 4, further comprising isolating or recovering the at least one identified recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance.